

SIV-Induced Instability of the Chimpanzee Gut Microbiome

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SUMMARY

Simian immunodeficiency virus of chimpanzees (SIVcpz) is the ancestor of human immunodeficiency virus type 1 (HIV-1), the etiologic agent of acquired immunodeficiency syndrome (AIDS) in humans. Like HIV-1-infected humans, SIVcpz-infected chimpanzees can develop AIDS-like symptoms. Because SIVcpz/HIV-1 may disrupt regulation of the gut microbiome and because it has not been possible to sample individual humans pre- and postinfection, we investigated the influence of infection on gut communities through long-term monitoring of chimpanzees from Gombe National Park, Tanzania. SIVcpz infection accelerated the rate of change in gut microbiota composition within individuals for periods of years after the initial infection and led to gut communities marked by high frequencies of pathogen-containing bacterial genera absent from SIVcpz-negative individuals. Our results indicate that immune function maintains temporally stable gut communities that are lost when individuals become infected with SIVcpz.

INTRODUCTION

Simian immunodeficiency virus (SIV) constitutes a class of lentiviruses detected in over 40 species of nonhuman primates (Chahroudi et al., 2012). SIVcpz, the SIV strain that infects chimpanzees, was transmitted to humans in the early 20th century (Korber et al., 2000), resulting in the global HIV-1 epidemic (Sharp and Hahn, 2011). In contrast to most SIVs, both SIVcpz and HIV type 1 (HIV-1) are pathogenic and can cause acquired immunodeficiency syndrome (AIDS) through the progressive depletion of host CD4⁺ T cells (Keele et al., 2009; Pantaleo et al., 1993). In particular, SIVcpz/HIV-1 infection decimates gut CD4⁺ T cells (Veazey et al., 1998), which help regulate the growth of bacteria within the gut microbiome (Slack et al.,

2009), and most HIV-1-infected patients experience some gastrointestinal dysfunction (Knox et al., 2000).

Despite the potential links between gut microbial communities and the progression of AIDS, the influence of SIVcpz/HIV-1 infection on the composition the gut microbiota remains poorly understood. To date, studies have been limited to comparisons of a few targeted bacterial taxa within infected versus uninfected individuals (Ellis et al., 2011; Gori et al., 2008). But given the high diversity within the gut microbiota and the substantial variation in gut microbiota composition among individuals (Arumugam et al., 2011; Moeller et al., 2012), a better approach would be to track the full composition of the gut microbiota within the same individuals before and after infection.

To test how SIVcpz infection affects the contents and stability of the gut microbiome, we followed the gut microbial communities of individual chimpanzees from Gombe National Park, Tanzania. Gombe chimpanzees represent the only habituated, wild-living ape population naturally infected by SIVcpz, and they have been monitored noninvasively for SIVcpz infection and AIDS-like symptoms for the past 13 years through the collection of fecal samples (Rudicell et al., 2010; Santiago et al., 2003; Terio et al., 2011). We identified six individuals who became infected during the observation period, two of which developed AIDS-like symptoms 3.5 and 4.5 years postinfection, while the others remained asymptomatic (Keele et al., 2009; Rudicell et al., 2010; Terio et al., 2011). We were thus able to track the composition of the gut microbiota in individuals who progressed from uninfected to SIVcpz positive, as well as from asymptomatic SIVcpz infection to AIDS.

RESULTS

SIVcpz Infection Distorts Gut Community Composition within Individuals

We sequenced bacterial 16S ribosomal RNA (rRNA) libraries prepared from 49 fecal samples collected over 9 years from 6 chimpanzees, yielding, per sample, an average of 11,260 high-quality sequences, which were binned into operational taxonomic units (OTUs) and assigned taxonomic classifications. The timings of fecal samples and SIVcpz infections across

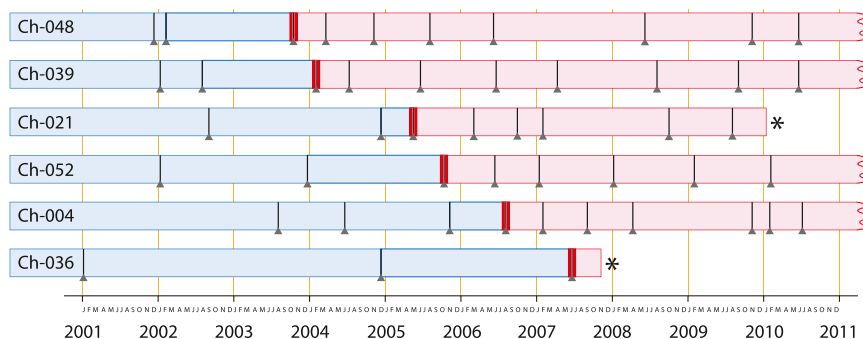


Figure 1. Timeline of Samples from Gombe Chimpanzees that Became Naturally Infected with SIVcpz

Horizontal bars correspond to individual chimpanzees whose gut microbiota were sampled during the past decade, with vertical slashes marking the time points at which fecal samples (triangles) were collected. Thick red verticals indicate the first sample for each chimpanzee in which SIVcpz was detected. Blue shading denotes periods when chimpanzees were uninfected, and red shading denotes periods after SIVcpz infection. Asterisks mark when infected chimpanzees died of AIDS-like symptoms; all other chimpanzees are still alive. Additional details about samples and hosts in Figure 1 are provided in Table S1. See also Figure S1.

individuals are presented in Figure 1. Complete sample information is presented in Table S1 (available online).

To determine whether SIVcpz infection altered the composition of the gut microbiota, we tested if samples obtained after infection differed from those recovered before infection in terms of the relative abundances of bacterial phylotypes. Based on Euclidean distances, which are well suited to detect changes in the frequencies of dominant community constituents, gut communities recovered from individuals postinfection differed significantly in composition from those present preinfection, and gut communities recovered from SIVcpz-infected individuals were significantly more variable in terms of community composition than were gut communities recovered from uninfected individuals ($p < 0.05$; Figure 2).

SIVcpz Infection Destabilizes Gut Community Composition within Individuals

The compositional divergence of the gut communities of SIVcpz-infected individuals could stem from one of two processes: first, SIVcpz infection could induce a single shift in gut community composition; second, SIVcpz infection could induce a prolonged instability in the gut community composition. To differentiate between these possibilities, we compared the degree of change between the communities recovered from consecutive samples from individual chimpanzees after SIVcpz infection with the degree of change between the communities recovered from consecutive samples from individual chimpanzees before SIVcpz infection. Gut microbial communities changed more over time after chimpanzees became infected with SIVcpz in terms of both the relative abundances ($p < 0.05$) and the presence and/or absence of bacterial phylotypes ($p = 0.05$) (Figure 3). This trend was evident despite longer average time intervals between consecutive SIVcpz-negative samples than between consecutive SIVcpz-positive samples.

SIVcpz Infection Increases Frequencies of Disease-Associated Bacterial Genera within the Gut Microbiome

After SIVcpz infection, gut communities displayed spikes in the relative abundances of pathogen-containing bacterial genera that were absent from individuals prior to infection. In particular, *Sarcina*, *Staphylococcus*, and *Selenomonas* rose substantially in relative abundance within several chimpanzees when they were infected with SIVcpz (Figure 4). Moreover, *Tetragenococcus*, a

bacterial genus that promotes T cell immunity (Masuda et al., 2008), rose in relative abundance within all six chimpanzees after they became infected with SIVcpz but was virtually absent before infection (Figure 4).

Stability of Core Bacterial Taxa within SIVcpz-Infected Individuals

Despite the effects of SIVcpz on the overall composition of the gut microbiota, no single OTU was consistently associated with SIVcpz infection (data not shown). Moreover, we observed stability in the composition of the gut microbiota at the phylum level across all sampled chimpanzees both before and after SIVcpz infection (Figure S1), and gut communities recovered from SIV-positive samples were no more diverse in terms of the numbers of phylotypes than those recovered from SIV-negative samples (Figure S2).

DISCUSSION

The long-term monitoring of chimpanzees from Gombe National Park, Tanzania revealed that SIVcpz infection distorted (Figure 2) and destabilized (Figure 3) the composition of gut microbial communities within individual hosts. The gut communities of SIVcpz-infected chimpanzees occupied a greatly expanded area of compositional space never accessed by the gut communities of uninfected chimpanzees (Figure 2). Moreover, SIVcpz led to elevated rates of change in the composition of the gut microbiota for years after the initial infection (Figure 3), even during periods when individuals appear healthy and exhibit no symptoms of AIDS. That SIVcpz infection appears to increase variation in gut microbiota composition both among individuals and within individuals over time is consistent with a scenario in which infection relieves constraints on the gut microbiome imposed by the host's immune system. This destabilization of the gut microbiota in response to SIV infection, detected through the longitudinal analysis of individual chimpanzees, parallels the increase in bacterial diversity observed in HIV-infected versus uninfected human cohorts (Lozupone et al., 2013).

The changes in the gut microbiota after infection with SIVcpz proceeded in an unpredictable manner across individuals: gut communities recovered post-SIVcpz infection contained high abundances of a variety of bacterial taxa that were rare within uninfected individuals, but these uncharacteristic taxa were not

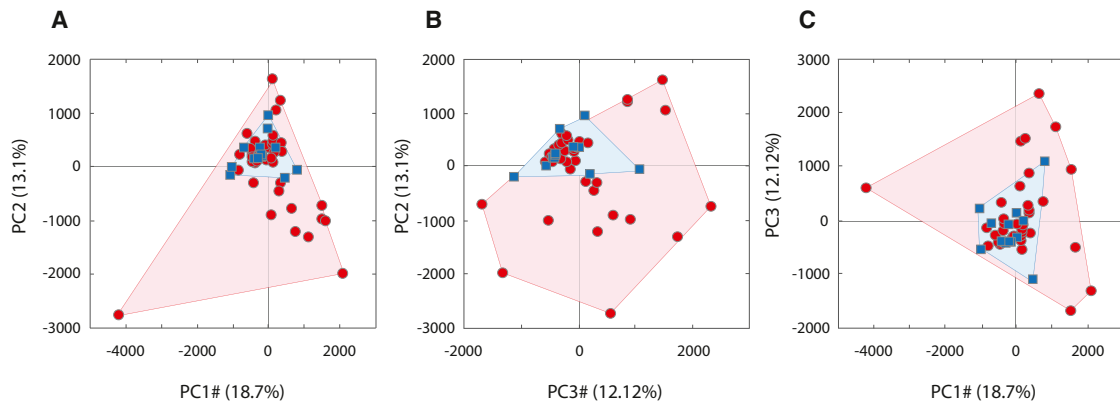


Figure 2. Compositional Divergence of SIV-Positive Gut Microbiomes

(A–C) Principal-coordinate plots of Euclidean distances among samples. Shown are all pairwise comparisons involving the first and second principal axes (A), the second and third principal axes (B), and the first and third principal axes (C). Together, the first, second, and third principal axes explain over 40% of the variance. Dots and surrounding contours correspond to gut communities recovered from individuals before (blue) and after (red) SIV infection.

identified in all SIVcpz-infected individuals. For example, *Tetragenococcus*, a bacterial genus that promotes T cell immunity (Masuda et al., 2008), constituted ~36%, ~8%, and ~0.5% of gut microbial communities recovered from chimpanzee hosts Ch-052, Ch-048, and Ch-039, respectively, after SIVcpz infection, and this genus was not found in any other samples from either SIV-infected or uninfected chimpanzees. Similarly, *Peptostreptococcus*, a genus of opportunistic pathogens that are a common cause of septicemia in humans (Murdoch, 1998), was detected in just two samples, both from Ch-021 post-SIVcpz infection.

Previous studies have reported enrichments of certain targeted taxa within the gut microbiota in HIV-1-infected cohorts, specifically in the frequencies of the orders Bacteroidales and Enterobacteriales (Ellis et al., 2011), which contain proinflammatory bacteria, and in the prevalence of the pathogen *Pseudomonas aeruginosa* (Gori et al., 2008). It has also been suggested that SIV/HIV may increase the diversity within the gut bacterial community (Saxena et al., 2012), as has been

observed in the gut viromes of SIV-infected monkeys (Handley et al., 2012). We did not observe associations between SIVcpz infection and the frequencies of Bacteroidales, Enterobacteriales, or *Pseudomonas*, nor did we observe an increase in alpha diversity within SIVcpz-positive chimpanzees (Figure S2). In fact, the phylum-level diversity of the gut communities within individual chimpanzees was relatively stable before and after SIVcpz infection (Figure S1). Moreover, the relative frequencies of bacterial phylotypes present within individuals before infection remained relatively stable after SIVcpz infection, and the abundance of no phylotype was associated with SIVcpz status. However, the genera *Sarcina*, *Staphylococcus*, and *Selenomonas* exhibited spikes in abundance when chimpanzees were infected with SIVcpz, blooming to relative frequencies as high as 30% (Figure 4). These genera contain opportunistic pathogens (Archer, 1998; Laass et al., 2010; Tanner et al., 1989) and were never detected at high abundances in the 35 SIVcpz-negative chimpanzees surveyed previously (Degnan et al., 2012). The link between SIVcpz infection and increases in the frequencies of disease-associated genera suggests that the distortion of gut microbiota induced by SIVcpz infection may pose health risks to hosts.

The long-term longitudinal sampling of chimpanzees in Gombe allowed us to track the composition of the gut microbiota before and after natural infections with SIVcpz, but also as individuals progressed from asymptomatic SIVcpz infection to AIDS. The gut microbiota of chimpanzees that manifested AIDS-like symptoms were not distinguishable from the microbiota of asymptomatic SIVcpz-infected chimpanzees (data not shown). CD4⁺ T cells are depleted in the gut during SIV/HIV infection long before the development of AIDS-like symptoms (Keele et al., 2009). Thus, the similarities between the gut microbial communities of individuals with symptomatic and asymptomatic SIVcpz infection are consistent with the depletion of host CD4⁺ T cells as a causative factor in the differentiation between the microbial communities of SIVcpz-infected and uninfected hosts.

In the two chimpanzees that developed AIDS (Ch-021 and Ch-036), there were increases in the frequencies of both *Sarcina* and *Staphylococci* OTUs in the final sample collected

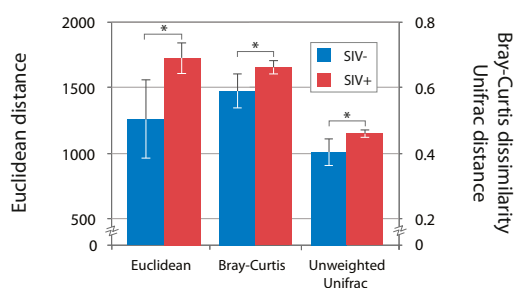


Figure 3. Temporal Destabilization of Gut Microbiomes after SIV Infection

Shown are pairwise distances and dissimilarities between consecutive samples recovered from individuals either before (blue bars) or after (red bars) SIV infection. Asterisks indicate statistically significant differences ($p < 0.05$), and error bars denote 95% confidence intervals for mean values. For all indices of dissimilarity and distance, gut microbiomes were less stable over time when individuals were SIV positive than when the same individuals were uninfected.

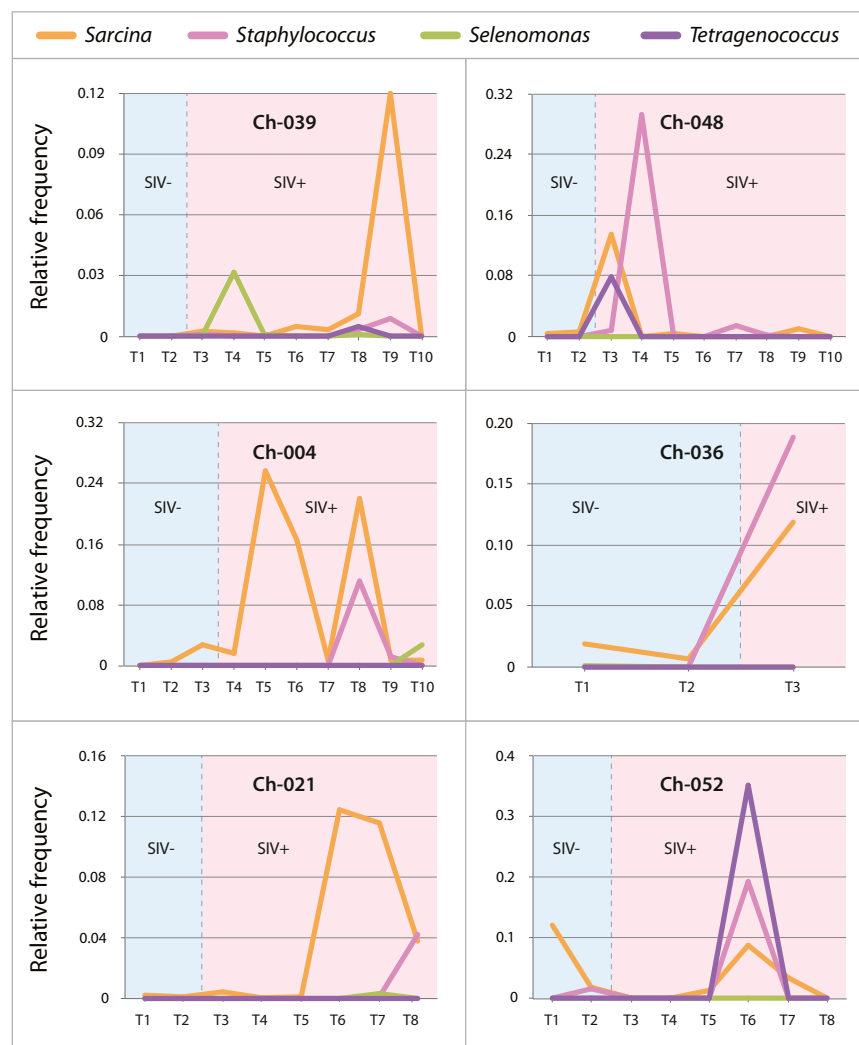


Figure 4. Increases in Frequencies of Bacteria from Disease-Associated Genera in SIV-Infected Chimpanzees

Shown are relative abundances of *Sarcina*, *Staphylococcus*, and *Selenomonas* pre- and postinfection. Graphs are partitioned into two sections denoting samples collected before (blue shading) and samples collected after (red shading) SIV infection. Longitudinal samples (T_n) are arranged temporally; dates of sampling shown in Figure 4 are listed in Table S1. See also Figure S2.

system, leading to a dysregulation of the gut microbiota. It has previously been reported that the loss of immune function caused by SIV/HIV can promote the systemic dissemination of *Salmonella* pathogens from the gut (Raffatellu et al., 2008). Our result that SIVcpz leads to major increases in the frequencies of a variety of potential pathogens further implicates the gut as a source of the opportunistic infections characteristic of AIDS. Moreover, the disruptive effects of SIVcpz infection on the gut microbiota are consistent with the observation that HIV-1-infected individuals suffer an increased risk for intestinal disorders (Knox et al., 2000) and highlight a need for longitudinal analyses of the influence of early HIV-1 infection on the human microbiome. In this context, the Gombe chimpanzees represent an invaluable resource because continuing to monitor infected and uninfected chimpanzees in these communities over time may reveal

before their deaths (Figure 4). Because these two samples were collected several years apart (Figure 1), the enrichments of these taxa occurred independently as these chimpanzees displayed AIDS-like symptoms. It will be important to determine whether *Sarcina* and *Staphylococci*, which also bloomed in the other SIVcpz-positive chimpanzees (who have remained asymptomatic as of this writing), might be an early predictor of subsequent immune deterioration.

By tracking the microbiota of individual chimpanzees that became naturally infected by SIVcpz during the course of natural history studies, we identified effects of SIVcpz infection on the gut microbiome that have previously gone unrecognized by comparisons of HIV-1-infected and uninfected humans (Ellis et al., 2011; Gori et al., 2008) or of SIV-infected and uninfected monkeys (Handley et al., 2012; McKenna et al., 2008). However, we were not able to explicitly correlate immune decline in SIVcpz-infected chimpanzees with gut microbiota composition, and as such, we cannot rule out the possibility that the changes in the gut microbiota we observed were mediated by other unmeasured factors. Nevertheless, our results suggest a progression in which SIVcpz infection deteriorates the immune

gut-microbiome-related predictors of host immune system decline.

EXPERIMENTAL PROCEDURES

Samples

A total of 49 fecal samples from 6 chimpanzees were selected from existing collections (Keele et al., 2009). Samples were originally collected between 2001 and 2010 and preserved in RNAlater (QIAGEN). In the field, trackers initially matched samples to hosts by individual recognition and later verified by mitochondrial and microsatellite markers (Keele et al., 2009). Unlike the sampling procedures employed for other wild SIVcpz-infected chimpanzee communities, fecal samples in Mitumba and Kasekela are collected under direct observation from known individuals. In this way, exposure times to the environment are minimal and very similar across samples. SIVcpz was detected in fecal samples from these individuals using a western blot to test for SIVcpz antibodies and RT-PCR using SIVcpz *Pts*-specific primers to test for the presence of SIVcpz sequences (Keele et al., 2009).

DNA Extraction and Purification

DNA was extracted using a modified bead-beating procedure. In short, fecal samples (100–200 μ l) were suspended in 710 μ l lysis buffer (200 mM NaCl, 200 mM Tris, 20 mM EDTA, 6% SDS) and 0.5 ml phenol/chloroform/isoamyl

alcohol (PCI) (pH 7.9), to which 0.5 ml 0.1 mm zirconia/silica beads were added. Cells were mechanically disrupted for 3 min in a bead-beater (BioSpec Products), centrifuged, and the aqueous phase was removed and subjected to a second PCI extraction. DNA was precipitated in an equal volume of isopropanol and 0.3 M sodium acetate (pH 5.5) and incubated overnight at -20°C . Precipitated DNAs were collected by centrifugation, and pelleted DNAs were washed with ethanol, air dried, and resuspended in 200 μl Tris/EDTA buffer (TE) (pH 8.0) containing 4 μg ribonuclease (RNase) A. Resuspended pellets were cleaned on QIAquick PCR Purification Columns (QIAGEN), and concentrated samples were adjusted to 25 ng/ μl for PCR amplification.

PCR Amplification and Sequencing

PCR primers that target the V6–V9 region of bacterial 16S rRNA were identical to those described by Degnan et al. (2012). PCR reactions were carried out in triplicate using forward primer TAXX-926F: 5'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG-NNNNNNNN-CT-aaa ctY aaa Kga att gac gg-3' and reverse primer TB-1492R: 5'-CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG TC-tac ggY tac cct gtt acg act t-3'. Capital letters correspond to the 454 sequencing primers, and the N's in the TAXX-926F forward primer denote the 8 nt barcode used to multiplex samples for sequencing in a single run. PCR reactions contained 15.8 μl PCR water, 2.5 μl ThermoPol Buffer (New England Biolabs), 2.5 μl 10 mM dinucleotide triphosphates (dNTPs) (5 PRIME), 1.5 μl of each 10 μM primer, 0.2 μl Taq Polymerase (New England Biolabs), and 1 μl template DNA. Reactions proceeded by incubation at 95°C for 2 min then were subjected to 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 90 s, with a final extension at 72°C for 10 min. Reaction success was verified by gel electrophoresis, and triplicate reactions were pooled and purified with AMPure XP Beads (Beckman Coulter). Samples were quantified using Quant-IT PicoGreen (Invitrogen), combined in equimolar amounts, and purified over a QIAquick Column (QIAGEN). Samples were sequenced using GS FLX Titanium Technology (Roche, 454 Life Sciences) to obtain 500 nt reads (University of Arizona Genetics Core).

Pyrotag Processing

Raw sequences were denoised and demultiplexed in QIIME v1.5.0 (Caporaso et al., 2010). Sequences outside the bounds of 460–1,000 nt, with an average error rate greater than 0.2% (quality score below 27) or with more than 1 error in the barcode were discarded. Remaining reads were trimmed to 460 nt. Using the QIIME pipeline, reads were aligned to the Greengenes core reference alignment (DeSantis et al., 2006) and taxonomically assigned using the RDP Classifier 2.2 (Wang et al., 2007). FastTree (Price et al., 2010) was used to construct an approximate maximum likelihood phylogenetic tree, and OTUs were picked at 99% similarity using uclust (Edgar, 2010). Chimeras were detected with ChimeraSlayer (Haas et al., 2011) using the Greengenes core alignment (DeSantis et al., 2006) as the reference set. Sequences that were identified as chimeric, chloroplast, or eukaryotic in origin, as well as those from OTUs with only a single representative read, were discarded.

Statistical Analyses

To compare samples, Bray-Curtis dissimilarities and Euclidean and UniFrac distances were calculated at an even depth of 7,000 randomly sampled sequences per sample. A one-tailed Student's *t* test for groups with unequal variances was used to test whether distances and dissimilarities between SIV-positive and SIV-negative samples were higher than those among SIV-negative samples, whether distances and dissimilarities between consecutive samples after SIV infection were higher than those between consecutive samples before SIV infection (Figure 3), and whether distances and dissimilarities among SIV-positive samples were higher than those among SIV-negative samples. G-test of independence and ANOVA were used to identify taxa over- or underrepresented within the SIV-positive fecal samples. Taxonomic stacked bar plots (Figure S1) were generated in QIIME v1.5.0. Rarefaction analyses (Figure S2) were performed in QIIME v1.5.0 using default settings.

ACCESSION NUMBERS

All sequence data for this project has been uploaded to the NCBI short-read archive under accession number SRR935433.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.chom.2013.08.005>.

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